Epidemiology of organomercury poisoning in Iraq. II. Relationship of mercury levels in blood and hair to exposure and to clinical findings

G. KAZANTZIS,[a] A. W. AL-MUFTI,[b] A. AL-JAWAD,[c] Y. AL-SHAHWANI,[c] M. A. MAJID,[c] R. M. MAHMOUD,[c] M. SOUFI,[c] K. TAWFIQ,[c] M. A. IBRAHIM,[c] & H. DABAGH[c]

In the survey described by Al-Mufti et al. (see page 23) blood and hair samples were analysed for total mercury by modified atomic absorption spectrophotometry. The hair samples were divided into 2.5-cm segments and analysed consecutively. The mean blood levels were 34 ng/ml and 7 ng/ml, respectively in those who had and those who had not eaten contaminated bread.

Corresponding mean maximum hair mercury values were 136 µg/g and 5 µg/g, respectively. Hair mercury values provided a better discrimination between different categories of exposure than blood mercury values at the time the survey was performed, some months after the end of the outbreak. Those persons who had not eaten contaminated bread but who lived in the area of high exposure had hair mercury values between the values of those who had eaten and those who had not eaten contaminated bread and who lived in the area of low exposure. Sequential estimation of mercury in 2.5-cm segments of hair in women gave information on the period of accumulation of mercury more than 1 year before the time of collection of the samples. It was possible to show an approximate relationship between the maximum hair mercury value and the amount of contaminated bread eaten. The match between the blood mercury level and the severity of poisoning was poor, owing to the length of time that had elapsed between the onset of poisoning and the sampling. With hair mercury, while the group results showed a good relation to the severity of poisoning, in individual cases the match was less good, especially in those persons where an insufficient length of hair was available for analysis. Biological variation in sensitivity to methylmercury was also likely to have been an important factor.

INTRODUCTION

Estimations of mercury concentration in blood and hair are of value in the investigation of the extent of exposure of a population to organomercury compounds. Clearance studies of methylmercury from blood have shown a biological half-life of approximately 70 days. Bakir et al.[1] in their study of 16 hospital patients in the outbreak under investigation showed a mean half-time of 65 days, but with a wide variation of 40-105 days. In their cases, the original blood levels were of the order of 1 000-4 000 ng/ml. The authors pointed out that large errors in epidemiological studies may result from an extrapolation of blood mercury levels back to the period of exposure. However, if an approximate half time clearance of 65 days is assumed, it could be expected that blood mercury levels would have fallen by about 3 times the half-life at the start of the survey

[a] Senior Lecturer, Middlesex Hospital Medical School, London, England.

[b] Director-General of Scientific Affairs, Ministry of Health, Baghdad, Iraq.

[c] Ministry of Health, Baghdad, Iraq.

of Hillali and Saglawiya, which has been described elsewhere (see page 27). The analytical method of Magos & Clarkson[2] is sufficiently simple and rapid for an epidemiological study and it was considered that it might also be sensitive enough to give information on exposure.

Analysis of sequential segments of hair were expected to provide a more powerful epidemiological tool, inasmuch as a time profile could be obtained extending backwards for 1 year or more if sufficiently long samples of hair were available. This was feasible in the adult female population but not in males or in children in rural Iraq. The same analytical method, modified for hair, was considered appropriate for an epidemiological study.

METHODS

The method of collection and analysis of the blood and hair samples for the survey has been described by Al-Mufti et al. (see page 26). It was decided to estimate only total mercury concentration in whole venous blood and in hair and to omit the selective estimation of inorganic mercury. Bakir et al.[1] have shown that only a small proportion of the mercury in blood in the present epidemic was present in the inorganic form. Little or no external contamination of hair with inorganic mercury compounds was likely to have occurred, as hair preparations were not used in rural Iraq and there was no evidence of occupational exposure to inorganic mercurials. Hair samples were cut into 2.5-cm lengths for analysis in sequence. For the ensuing calculations a uniform average rate of growth of hair of 1.13 cm per month was assumed for both sexes. Results of hair analysis are given for the first 2.5 cm of hair, i.e., the growing end close to the scalp, and also for the maximum value in the whole length of hair analysed. The former measure relates to exposure in the period immediately prior to sampling, whilst the latter can be related to the period when maximal accumulation had occurred.

RESULTS

The survey included 473 people over the age of 5 years who had admitted to eating contaminated bread and 1 267 persons who claimed that they had not done so. Blood samples were taken from 88% and 80% of these groups respectively, and hair samples from 82% and 92% respectively (Table 1(I)). The mean blood mercury level was 34 ng/ml in those who had eaten contaminated bread and 7 ng/ml in those who had not done so. Corresponding maximum hair mercury values were 136 μg/g and 5 μg/g. Thus, the current blood mercury level at the time of the survey was 5 times as high in those who had eaten contaminated bread, but the maximum hair mercury level in this group exceeded the level in those who had not eaten by a factor of 27.

Persons of all ages in the survey population were subdivided into 4 exposure categories as follows:

Category 1: Persons who had been hospitalized for mercury poisoning.

Category 2: Members of the same household as those in Category 1, nearly all of whom had eaten contaminated bread, and other persons in the affected area who had eaten contaminated bread.

Category 3: Other persons living in the area of high exposure who stated that they had not eaten contaminated bread.

Category 4: Persons living in the area of low exposure.

TABLE 1. MERCURY LEVEL IN BLOOD AND MAXIMUM MERCURY LEVEL IN HAIR IN THE SURVEY POPULATION

Group	Number examined	Mean blood Hg level ng/ml	S.E.[a]	Number examined	Maximum hair Hg level µg/g	S.E.[a]	Age group
(I) Contaminated bread eaten or not							
Ate contaminated bread	413	34	5.1	385	136	17.8	>5 years
Did not eat contaminated bread	1 012	7	0.9	1 160	5	0.8	
(II) Exposure category[b]							
1	126	42	7.4	118	275	54.2	
2	375	25	5.1	355	71	8.4	All ages
3	418	7	1.4	445	12	2.0	
4	767	5	1.1	963	1	0.4	
(III) Diagnostic grouping							
Clinical mercury poisoning:							
Severe disability	29	53	14.9	27	180	83.7	
Mild/moderate disability	36	25	7.9	33	128	33.5	All ages
Subjective poisoning only	129	34	7.9	115	211	45.0	
No evidence	1 485	10	1.4	1 699	18	2.7	

[a] Standard error of the mean.

[b] For definition, see text.

All those in Category 1, and all but 12 of those in Category 2 (3.3%) had eaten contaminated bread. Subsequent analysis showed that, in Category 3, 2 people had in fact eaten bread containing mercury, but none in Category 4 had done so. The mean level of mercury in blood and hair, together with the standard error of the mean in these 4 categories, is shown in Table 1(II). For both blood and hair, mean mercury levels were lowest in those living in the area of low exposure and rose in order with the category of exposure. Hair values provided a better discrimination between the categories than blood values. For example, for Categories 3 and 4 the difference between the means for hair mercury was highly significant ($\underline{t} = 6$; $p < 0.01$) but not significant for blood mercury ($\underline{t} = 1.9$).

In the 4 diagnostic groups already defined (see page 25) mean blood and hair mercury levels were examined (Table 1(III)). The discrimination between these groups was less clear than expected because the groups with disability were small and the means were influenced by factors such as the time of collection of the sample relative to the onset of poisoning, and availability of a sample. For example, in the severe disability group mean blood mercury for the 18 samples collected before the end of February 1973 was 84 ng/ml, and the 11 blood samples in the mild/moderate disability group averaged 73 ng/ml. Blood mercury levels were very low in samples taken later. As regards the mean values for maximum hair mercury, there were only 6 persons, all females, in the severe disability group who could provide more than a 5-cm length of hair and for these the mean maximum value was 662 μg/g. In the mild/moderate disability group, 17 persons out of 33, all but one of whom were female, were able to provide more than a 5-cm length of hair. In these 17 persons the mean maximum hair mercury concentration was 233 μg/g. Provided account is taken of these factors, therefore, a relationship could be shown between the severity of the clinical picture of mercury poisoning and the group mean values for total mercury in blood and in hair.

Table 2 shows the mean blood level and the mean maximum hair level for mercury, subdivided according to the amount of contaminated bread, in loaf/days, that had been eaten. It was not possible to show a relationship between the blood mercury level and the amount of bread eaten. However, the maximum value for mercury in hair differentiated those who had eaten more than 100 loaves from those who had eaten less by a factor of 2.5.

TABLE 2. MEAN BLOOD MERCURY AND MEAN MAXIMUM HAIR MERCURY LEVELS RELATED TO THE TOTAL AMOUNT OF CONTAMINATED BREAD EATEN

Estimated bread eaten (loaves x days)	Mean blood mercury level		Mean maximum hair mercury level	
	No.	ng/ml	No.	μg/g
1 - 49	57	38	62	90
50 - 99	147	38	134	88
100 - 199	108	32	96	194
200 - 499	67	41	61	259

Tables 3 and 4 show the distribution of blood and of maximum hair mercury values in the survey population over the age of 5 years, divided into different exposure categories: (i) those who lived in the area of low exposure, none of whom had eaten contaminated bread; (ii) those who lived in the area of high exposure but had not done so; (iii) those who had eaten contaminated bread; and (iv) those who had done so and had previously been hospitalized. Whilst 10% and 12%, respectively, of those in the areas of low and high exposure

who had not eaten contaminated bread and blood levels of mercury above 10 ng/ml, the proportion of the exposed group with these levels rose to 21% for those who had done so and to 50% for those who had done so and had previously been hospitalized.

TABLE 3. DISTRIBUTION OF BLOOD MERCURY VALUES IN THOSE WHO HAD NOT EATEN AND THOSE WHO HAD EATEN CONTAMINATED BREAD

Blood Hg (ng/ml)	Percentage of total			
	Saglawiya	Hillali		
	Bread not eaten	Bread not eaten	Bread eaten	Bread eaten, and person previously hospitalized
< 10	90	88	79	50
10 - 49	8	9	12	27
50 - 99	<1	1	2	11
100 - 199	<1	1	4	6
200 - 299	0	0	<1	3
300 - 399	<1	<1	<1	2
400 - 499	<1	0	<1	2
500 and above	0	0	<1	0
Total (persons)	767 (100%)	311 (100%)	319 (100%)	124 (100%)

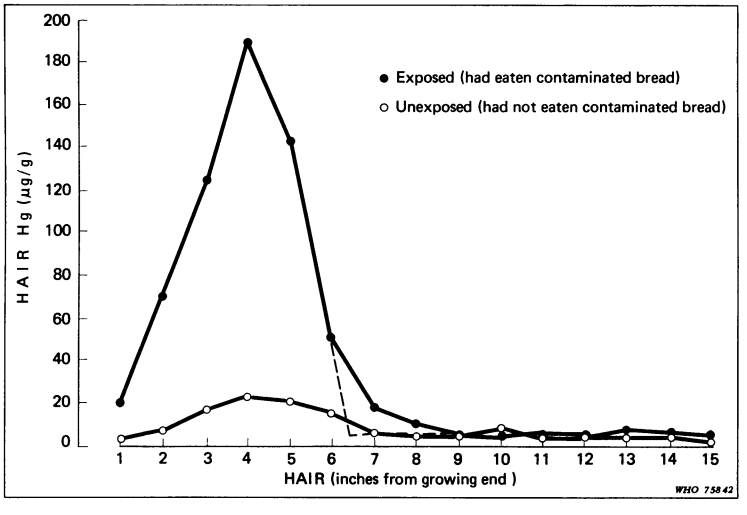
TABLE 4. DISTRIBUTION OF MAXIMUM HAIR MERCURY VALUES IN THOSE WHO HAD NOT EATEN AND THOSE WHO HAD EATEN CONTAMINATED BREAD

Maximum hair Hg (µg/g)	Percentage of total			
	Saglawiya	Hillali		
	Bread not eaten	Bread not eaten	Bread eaten	Bread eaten, and person previously hospitalized
<1	88	43	23	13
1 - 9	10	32	19	15
10 - 40	1	16	20	18
50 - 99	<1	6	12	9
100 - 199	<1	2	16	19
200 - 299	0	1	5	8
300 - 399	<1	<1	3	3
400 - 499	0	1	2	2
500 and above	0	0	1	13
Total (persons)	963 (100%)	326 (100%)	306 (100%)	118 (100%)

With regard to maximum hair mercury, 2% of the low exposure group, 25% of those in the high exposure group who had not eaten contaminated bread, 58% of those who had done so and 72% of those hospitalized had values above 10 μg/g. It can be seen that hair mercury differentiates these exposure groups very clearly. The 326 subjects who lived in the high exposure area and who claimed not to have eaten contaminated bread had a distribution pattern for hair mercury intermediate between that of the low exposure group and that of the group that had eaten contaminated bread.

Fig. 1 shows the mean total mercury concentration in successive 2.5-cm segments of hair in females in those who had eaten and those who had not eaten contaminated bread. In both groups the mean mercury values are low in the distal part of the hair and these give an indication of the pre-exposure baseline. In the exposed group there is a very rapid rise in mercury concentration to a peak value, which indicates the period of accumulation of mercury in the body, followed by what appears to be an almost equally rapid decline. In those who had not eaten contaminated bread, there is a small rise, reaching a maximum value at approximately the same distance from the growing end of the hair as in the other group, the concentration then falling to its pre-exposure value by the first 2.5 cm. In terms of the time at which these fluctuations occurred, a mean hair growth rate of 1.13 cm per month was assumed for Arab women of all ages, this being the mean of 41 observations by Al-Shahristani & Shihab.[3] The variation given by these authors was within a range of 0.90-1.5 cm and is in accordance with published figures for hair growth rate in other countries. The beginning of the accumulation period was determined, by the method of Giovanoli-Jakubczak,[4] as 14.5 months before the mean time for sampling. This is in accord with the history of the eating of contaminated bread, going back to the period around the end of Ramadan 1971.

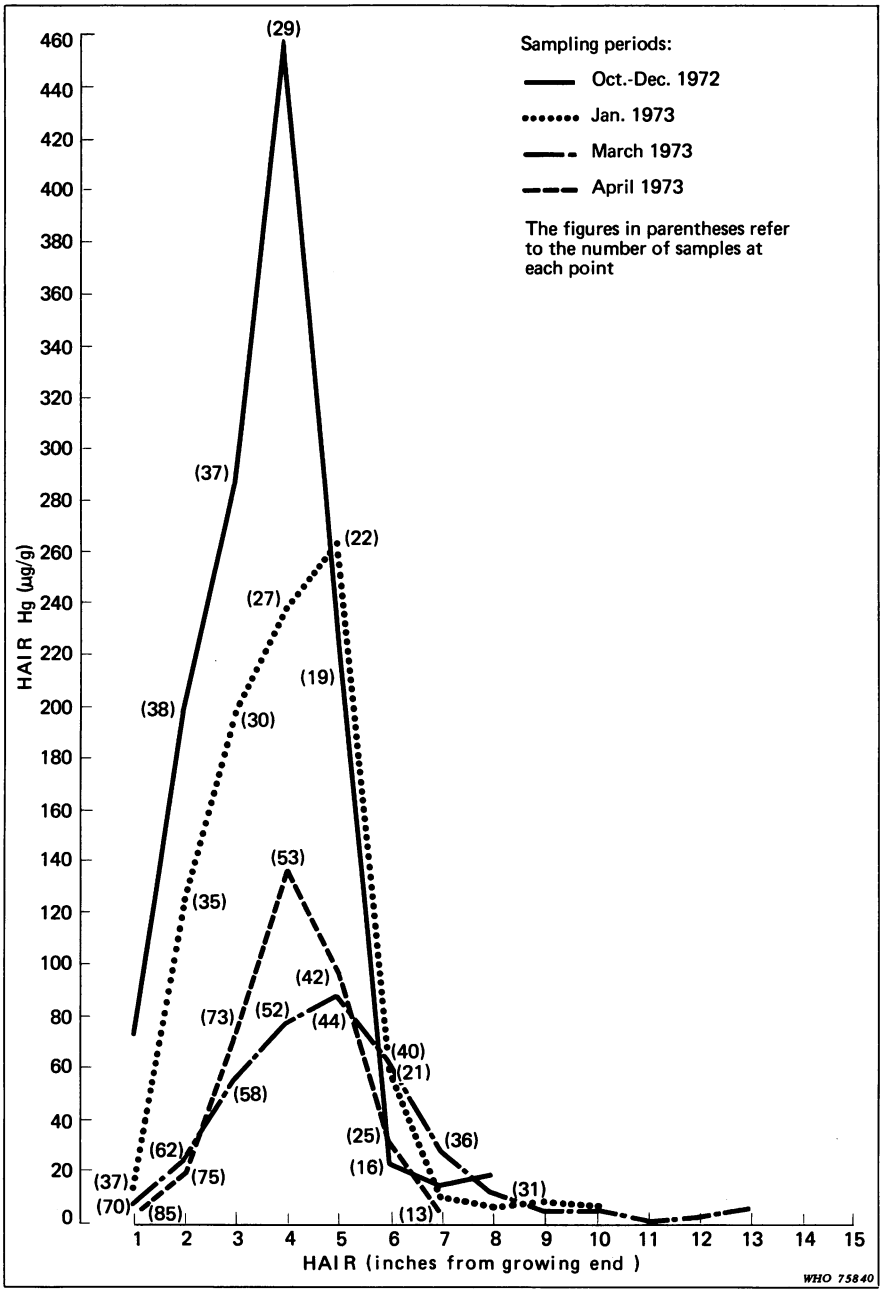
FIG. 1. MEAN HAIR MERCURY IN FEMALES



The curve showing the profile of exposure to mercury had been plotted from the mean mercury concentration in hair at 2.5-cm intervals from a large number of subjects; for example, the first, third, and fifth 2.5-cm values were derived from the means of 250, 210, and 133 observations, respectively. However, these samples were taken at different times during the survey period, from October 1972 to the end of April 1973. Mean curves were also derived for hair mercury concentration according to the month of sampling (Fig. 2). As few samples were collected from October to December, the data for these three months were pooled and plotted together with the data for the months of January to April. The peak values fell markedly in succeeding months, but the significance of this is difficult to interpret as there was a tendency for the more severely affected to be seen earlier in

the survey. It would be expected that the time interval from the time of sampling to the beginning of the accumulation period, as shown by the curves, would progressively lengthen as the survey progressed. This time interval was calculated at 13 months for the October to December samples, 14 months for those collected in January, and 17, 17, and 14 months for the samples collected in February, March, and April, respectively.

FIG. 2. MEAN HAIR MERCURY LEVELS IN FEMALES WHO HAD EATEN CONTAMINATED BREAD



Similar curves for those who had not eaten contaminated bread but who lived in the exposed area of Hillali are shown in Fig. 3. These curves appear less regular than those for persons who had been heavily exposed but which are based on smaller total estimations. Here too the peak values for the earliest sampling period were by far the highest, but this cannot be explained on the grounds that the more severly affected persons were seen first. Fig. 4 shows the mean hair mercury curve for those women in the area of high exposure who had not eaten contaminated bread, compared with the corresponding curve for the area of low exposure. Whilst an appreciable increase during the relevant period could be shown in the former, in the area of low exposure no increase in mean hair mercury could be demonstrated.

The diagnosis of mercury poisoning in this survey was made on clinical grounds and the categories have been discussed elsewhere (see page 26). In individual cases, the blood and hair mercury values obtained did not correlate well with the severity of the clinical picture. The highest blood mercury values in the severe disability group, in ng/ml, were 320 (collected October) and 220 (collected December); in the mild/moderate disability group, 180 (collected October), 120 (collected December), and 120 (collected March); and in the subjective group 470 (collected October), 520 (collected October), and 490 (collected December). However, there were altogether 4 results for blood mercury which exceeded 500 ng/ml; the other 3 high levels were found in people who were without clinical evidence of poisoning. Two people in the groups with disability had blood mercury below the level of detection before March 1973, but from March onwards most blood mercury levels were unrecordable.

FIG. 3. MEAN HAIR MERCURY LEVELS IN FEMALES LIVING IN HILLALI OR SURROUNDING AREA WHO HAD NOT EATEN CONTAMINATED BREAD

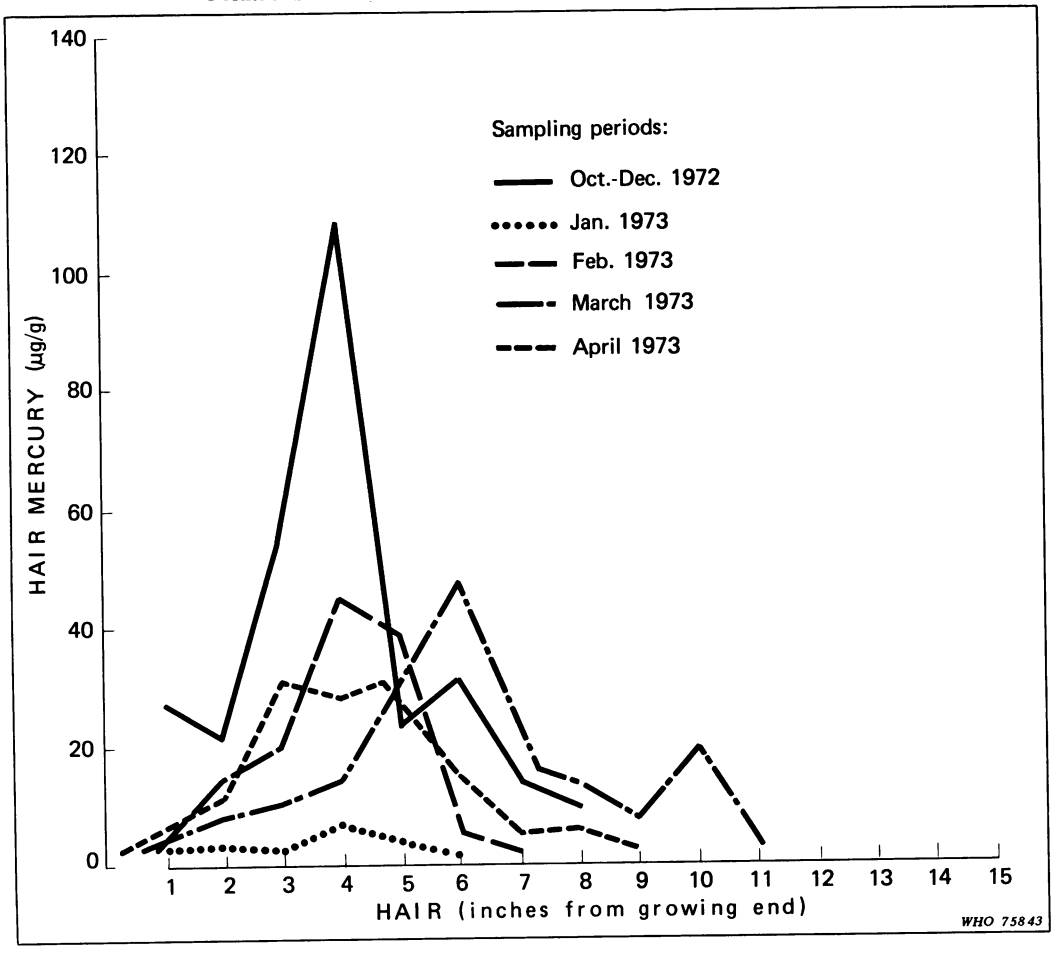
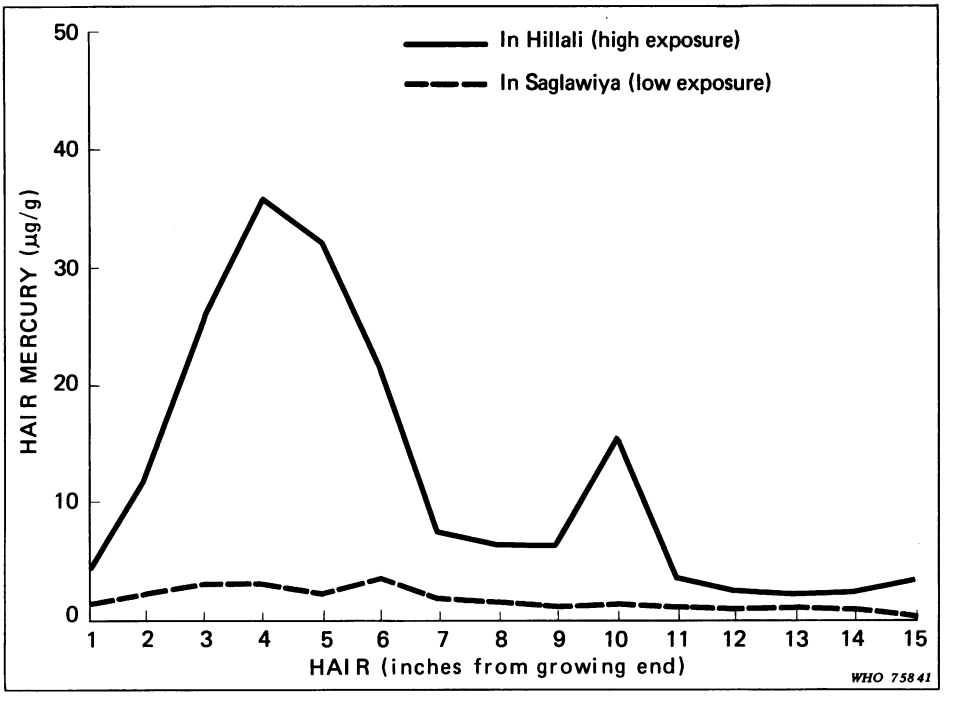


FIG. 4. MEAN HAIR MERCURY LEVELS IN FEMALES WHO HAD NOT EATEN CONTAMINATED BREAD



The highest values of hair mercury in the severely disabled group were 2 130 μg/g (third 2.5 cm), 890 μg/g (third 2.5 cm), and 360 μg/g (fourth 2.5 cm). In the mild/moderate group the highest values were 860 μg/g (second 2.5 cm), 430 μg/g (third 2.5 cm), and 550 μg/g (fifth 2.5 cm); and in the subjective group 3 300 μg/g (fifth 2.5 cm), 3 000 μg/g (fourth 2.5 cm) and 1 300 μg/g (fourth 2.5 cm). In each of these clinical groups the lowest figures for hair mercury were below the limit of detection.

In other published series the mercury concentration in the growing end of the hair has been shown to be 200-300 times the concentration in blood. This constant relationship has not been observed to apply to individuals in this series, but has been found to apply for group results. Thus, for 1 452 nonexposed subjects, the ratio of the proximal mercury level in hair to blood was 267:1. For the exposed group, this ratio was 434:1.

DISCUSSION

It would have been preferable to have processed 1-cm lengths of hair instead of 2.5-cm lengths, as these would have isolated the peaks of mercury concentration more precisely. However, even in 1-cm lengths, the result is an average along that length and does not represent the actual peak obtained. In view of the number of variables discussed below, it is not considered that the results suffered from the use of 2.5-cm lengths, provided it is remembered that this was an epidemiological study which stresses the relationship between the results for different groups rather than the actual figures obtained.

A population study to include clinical and analytical data in an area where heavy exposure to organomercury compounds had occurred some time previously has not been described before. Differentiation between those who had eaten contaminated bread and those who had not done so was possible on a group basis with both blood and hair mercury. Hair mercury levels were also of value in differentiating between those people who lived in the areas of high and of low exposure, and all of whom claimed not to have eaten contaminated bread. The long hair of female subjects was of particular value in this respect, as it enabled a time profile of exposure to mercury to be constructed, going back for almost 18 months from the time of the study. There are several variables which render the exact interpretation of these mercury exposure profiles difficult.

The time interval between the end of exposure and the sampling was variable, since people stopped eating contaminated bread at different times between December 1971 and February or March 1972, and samples were taken from October 1972 to the end of April 1973. The total period of exposure also varied, as did the beginning of exposure, being centred around the end of Ramadan 1971. In spite of these variable factors the mercury profile curves for hair show the beginning of the accumulation period to have been in November 1971, and some lengthening of the interval from this point to the time of sampling has been demonstrated in the later stages of the survey. Determination of the mercury content in consecutive segments of hair in females therefore appears to be of considerable value in the surveillance of a population exposed to this metal.

There are various possible reasons for the imperfect relationship between the individual values for hair or blood mercury and the clinical picture. It was not possible to relate the hair mercury level in male patients to their degree of clinical involvement as with their short hair the clinical picture could not be related to the hair mercury level at the time of maximal accumulation. Some unexpectedly high individual values for mercury in hair in otherwise normal persons may have been artefacts. It is possible that external contamination of hair may have occurred as a result of sowing dressed grain, or of visiting the mills where the contaminated wheat had been ground. The rural population do not wash their hair frequently, and the hair samples were not washed before analysis. Again, some atypical values for both hair and blood mercury

may have been due to random errors of identification, analysis, recording, or transcription, which should be allowed for in a survey of this size. Even allowing for these factors, however, the possibility must be considered that biological variation was responsible for some of this lack of relationship with the clinical picture.

The detection of an elevated blood mercury level in the survey depended on the initial level of mercury in the blood, on its half-time in the blood and on the time interval before sampling. Approximately 3 half-times had elapsed from the apparent end of the epidemic to the start of the survey, and another 3 half-times to its end. The pattern of blood mercury values showed a marked fall to undetectable levels in the later stages of the survey. While many but not all of the more severe cases were seen early in the survey, the fall in blood mercury over the survey period was one likely important reason for its variation in relation to the clinical picture, which itself changed with time but probably not to the same degree as the mercury level. The variation between individuals in the biological half-life, which has been studied by Al-Shahristani & Shihab[3] and by Bakir et al.[1] and shown to be of considerable magnitude, was another factor to be considered. Finally, account should be taken of the variation in susceptibility to all toxic agents which occurs as between individuals. In the field visits, families were seen, some members of which had suffered all degrees of severity of mercury poisoning, ranging from death and severe disability to symptomatic involvement only, while other members of the same family appeared not to have been affected. It is unlikely that the intake of mercury in these families could have been varied so much from one member to another as to be entirely responsible for this observed difference in clinical effect.

RESUME

EPIDEMIOLOGIE DES INTOXICATIONS PAR ORGANOMERCURIELS EN IRAK

II. RAPPORT DE LA CONCENTRATION DE MERCURE DANS LE SANG ET LES CHEVEUX AVEC LE DEGRE D'EXPOSITION ET LES MANIFESTATIONS CLINIQUES

Lors de l'enquête décrite par Al-Mufti et al. (voir p.23) on a utilisé la spectrophotométrie d'absorption atomique modifiée pour déterminer la teneur en mercure d'échantillons de sang et de cheveux. Les échantillons de cheveux ont été divisés en segments de 2,5 cm et analysés successivement. Dans les échantillons de sang, les concentrations observées ont été de 34 ng/ml et 7 ng/ml respectivement chez les sujets qui avaient consommé du pain contaminé et chez ceux qui n'en n'avaient pas consommé.

Les chiffres correspondants pour les échantillons de cheveux ont été respectivement 136 µg/g et 5 µg/g. Les concentrations de mercure dans les échantillons de cheveux ont permis de mieux différencier les degrés d'exposition que les concentrations dans le sang au moment de l'enquête, c'est-à-dire plusieurs mois après la fin de l'épisode. Les personnes n'ayant pas consommé de pain contaminé, mais habitant dans la zone de forte exposition, présentaient des concentrations de mercure dans les cheveux qui se situaient entre les concentrations observées chez les personnes ayant consommé et chez les personnes n'ayant pas consommé de pain contaminé dans la zone de faible exposition. Le dosage du mercure dans des segments de cheveux de femme de 2,5 cm a fourni des renseignements sur la période d'accumulation du mercure pendant plus d'un an avant le moment de la collecte des échantillons. Il a été possible de mettre en évidence une relation approximative entre la concentration maximale de mercure dans les cheveux et la quantité de pain contaminé consommée. La relation entre la concentration de mercure dans le sang et la gravité de l'intoxication s'est révélée assez médiocre, cela à cause du temps écoulé entre le moment de l'intoxication et celui de l'échantillonnage. Dans le cas des échantillons de cheveux, si les résultats d'ensemble ont fait apparaître une assez bonne

correlation entre la concentration de mercure et la gravité de l'intoxication, la concordance a été moins bonne dans les cas individuels, particulièrement chez les personnes sur lesquelles il n'a pas été possible de prélever des échantillons de cheveux de longueur suffisante. La variation biologique de sensibilité aux composés méthylmercuriels a pu jouer aussi un rôle important.

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